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# IMMUNOHISTOCHEMICAL DETECTION OF NAD(P)H:QUINONE OXIDOREDUCTASE 1 IN HUMAN BONE MARROW.

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NAD(P)H:quinone oxidoreductase 1 (NQO1) is a cytosolic two-electron reductase that catalyzes the reduction of quinones to hydroquinones. A polymorphism in NQO1 has been characterized (NQO1\*2), a C to T substitution at position 609 of the human NQO1 cDNA that codes for a proline to serine amino acid change at position 187 of the protein. Genotype-phenotype studies have shown those individuals homozygous for the NQO1\*2 polymorphism are deficient in NQO1 due to enhanced proteasomal degradation of the mutant protein. Epidemiological data from benzene exposed workers has demonstrated an increased risk of developing hematological malignancies with the homozygous NQO1\*2 genotype in agreement with the proposed protective role of NQO1 against benzene derived quinones. No NQO1 protein, however, could be detected in freshly isolated human bone marrow aspirates by immunoblot analysis which was inconsistent with the proposed protective mechanism for NQO1. In this study we have performed immunohistochemical analysis of human bone marrow sections from B-5-fixed, decalcified, paraffin-embedded human bone marrow core biopsies which revealed that NQO1 is expressed in human bone marrow but expression is limited to endothelial cells and adipocytes. Immunostaining for NQO1 was detected in the endothelium of large blood vessels as well as endothelium of the bone marrow microvasculature. NQO1 immunostaining in adipocytes was greatest in cells adjacent to vasculature. These data demonstrate that NQO1 is present in human bone marrow and may protect cell populations in bone marrow from xenobiotic-induced toxicity (supported ES09554).

# PLASMA FATTY ACID ETHYL ESTERS AND BLOOD ALCOHOL CONCENTRATION IN ALCOHOLICS.

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After biliary duct diseases, chronic alcoholism is the major cause of pancreatitis. Metabolism of ethanol to fatty acid ethyl esters (FAEEs) via conjugation of ethanol with endogenous fatty acids appears to be one of the major pathways of ethanol disposition in the pancreas during chronic alcohol abuse, and could be associated with ethanol-induced pancreatic damage. Although FAEEs are shown to be toxic in vivo and in vitro, their involvement in ethanol-induced diseases is not well understood. In most cases, pancreatic damage occurs preceding the onset of clinical pancreatitis in alcoholics. Therefore, determining indicators of ethanol-induced diseases in the plasma could be important for an early diagnosis and prevention of diseases associated with alcohol abuse. In the present study we measured plasma alcohol and FAEE levels in 39 hospital patients with the history of acute and chronic ethanol exposure. Six individuals were identified with acute intoxication and 5 with chronic alcoholism from their case history. The remaining cases were alcoholic, but with no well defined alcohol abuse history. Significantly high level of alcohol and FAEEs were found in the plasma of all the alcoholics than those in controls. The FAEE levels (ranged 265-43,120 ng/ml) were linearly related to the blood alcohol concentration and were 4 fold higher in chronic alcoholics than those with acute ethanol exposure. Apart from 16:0 and 18:1 FAEEs, detected mostly in all the samples, 18:0 and 18:2 FAEEs were also detected more frequently in the chronic alcoholics. A correlation between the levels of FAEEs in the plasma and those, which cause pancreatic injury, can be of diagnostic importance.

# VARIATION IN N-ACETYLTRANSFERASE 2 ACTIVITY AND 4-AMINOBIIPHENYL GENOTOXICITY.

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N-Acetyltransferases catalyze the acetylation of aromatic amines. Mammalian species have two genes, NAT1 and NAT2. In humans, polymorphisms in both genes result in phenotypic variation. Low NAT2 activity is associated with increased risk of aromatic amine-induced urinary bladder cancer and with higher levels of carcinogen-hemoglobin (Hb) adducts. The hypothesis that lower NAT activity increases the genotoxicity of aromatic amines was investigated in mice. C57Bl/6 mice are rapid acetylators with the NAT2\*8 allele. A/J mice, slow acetylators, have the NAT2\*9 allele and the congenic B6.A strain has the NAT2\*9 allele on the C57Bl/6 background. All three strains have the same NAT1. Adult male mice received a single oral dose of 120 mg 4-aminobiphenyl (4ABP)/kg or corn oil. After

24h, livers were harvested and frozen. Histological sections (4-5µm) were prepared and 4ABP-DNA adducts detected by immunofluorescence. Increased nuclear fluorescence was observed in all three strains. There was no significant variation by strain. Hepatic NAT activity was measured with the murine NAT 2 selective substrate p-aminobenzoic acid (PABA) and with 4ABP. Hepatic PABA NAT activity in C57Bl/6 was 7.74±0.13 nmol acetylPABA/min/mg, 3.05±0.58 for A/J and 4.19±0.58 for B6.A. No significant differences were observed in 4ABP NAT activity. Values ranged from 1.1±0.34 to 1.52±0.24 nmol acetyl4ABP/min/mg. NAT2 genotype correlated with phenotype for PABA acetylation, but there was no phenotype-genotype correlation with 4ABP. The lack of strain variation in 4ABP NAT activity was consistent with the comparable 4ABP-DNA adduct levels observed in the three strains. The association between NAT2 phenotype and 4ABP-Hb adducts in humans was not seen in mice. Substrate selectivity and amino acid sequence support the functional analogy of human NAT2 with murine NAT1 and vice versa. The contribution of murine NAT1 to 4ABP genotoxicity remains to be elucidated. (ES09812, ES10047 (CAM) and ES08846 (MAP)).

# THE RELATIONSHIP BETWEEN PON1-192 GENOTYPE AND PHENOTYPE IN HUMAN LIVER.

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Individuals vary widely in their toxic response to phosphorothioate (OPs) pesticides. This may be influenced by the individuals' capacity to activate and detoxify (metabolise) these compounds. OPs are metabolised via the cytochrome P450/paraoxonase (PON1) pathways. PON1 is polymorphic at position 192 in the human population. The R (mutant) isoform hydrolyses paraoxon (POX) rapidly, while the Q (wild type) isoform hydrolyses it slowly. Both isoforms hydrolyse chlorpyrifos oxon (CPO) and phenylacetate (PA) at the same rate. Our previous studies with human liver showed that parathion and chlorpyrifos were activated to their oxons with 15-fold and 10-fold ranges in activity. The OPs were mainly activated by CYPs 3A4/5 and 2C8, however, the PON1 pathway was more important for chlorpyrifos metabolism. This study investigated the capacity of 27 human liver microsomal preparations to metabolise POX and CPO. DNA was extracted from the livers and the PON1 genotype for the 192 polymorphism was determined by PCR amplification followed by SSCP (single-stranded conformation polymorphism) analysis. Inspection of the SSCP showed that 7 individuals (26%) were mut/mut (RR), 13 were wt/wt (QQ, 48%) and the remainder (26%) were QR for PON1. The POXase (0.17-9.6nmol/min/mg protein) and CPOase (17.9-288 nmol/min/mg protein) activities were correlated with phenylacetate hydrolysis (4.1-19.9µmol/min/mg protein). These plots revealed a wide range in metabolic capacity within each of the genotypes indicating that both genotype and phenotype are important determinants of an individual's PON1 status. This work was partly funded by the Colt Foundation

# IN VITRO METABOLISM OF PYRIDOSTIGMINE BROMIDE (PB), DEET AND PERMETHRIN, ALONE AND IN COMBINATION BY HUMAN PLASMA AND LIVER MICROSOMES.

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PB, DEET and permethrin alone, or in combination were incubated in human plasma or liver microsomes at 37°C for 60 min. DEET was further incubated in human plasma for 8 h period. N-methyl-3-hydroxypyridinium bromide was detected as a metabolite of PB in plasma with  $K_m$  and  $V_{max}$  of 47µM and 15.3pmol/min/mg of protein, respectively. DEET metabolism in plasma was very slow either after 1 or 8 h period of incubation. Disappearance of permethrin following incubation with human plasma was very rapid.  $K_m$  and  $V_{max}$  values for the conversion of permethrin to m-phenoxybenzyl alcohol were 73µM and 142.3pmol/min/mg of protein, respectively. The selective butyrylcholinesterase inhibitor tetraisopropyl pyrophosphoramidate (Iso-OMPA) significantly inhibited permethrin metabolism in human plasma. The 50% inhibitory concentration ( $IC_{50}$ ) of (Iso-OMPA) for the formation of the permethrin metabolite m-phenoxybenzyl alcohol was estimated to be 91mM. Metabolism of PB in human liver microsomes was negligible. m-Toluamide and m-toluic acid were identified as metabolite of DEET in human liver microsomes incubates. Permethrin metabolites m-phenoxybenzyl alcohol and m-phenoxybenzoic acid were detected after 60 min of incubation in human liver microsomes. Rate of DEET metabolism was slower following combined incubation of DEET and permethrin in liver microsomes compared to individual chemical. These findings indicate that esterases and oxidases are involved in

metabolism of PB, DEET, and permethrin and that PB and permethrin are metabolized mostly by plasma butyrylcholinesterase, while DEET is mainly metabolized by oxidase enzymes. This study was supported in part by the U.S. Army Medical Research and Material Command under Contract No. DAMD 17-99-1-9020.

#### 430 PROTECTION AFFORDED BY HEPATIC A-ESTERASE TO ORGANOPHOSPHATE TOXICITY IN JUVENILE AND ADULT RATS.

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Juvenile rats are more sensitive to the acute toxicity of organophosphate (OP) insecticides than are adults. The lower levels of hepatic A-esterase activity in young animals may contribute to the greater toxicity of these compounds in juveniles. Indirect A-esterase analysis toward low physiologically relevant concentrations of diazoxon, azinphosmethyl-oxon, O-methoate, dichlorvos and coroxon were assayed in whole liver homogenate in the presence of calcium (a cofactor for A-esterase) or EDTA. Bovine brain homogenate was used as an exogenous source of cholinesterase [ChE] to trap unhydrolyzed oxon. Incubation of azinphosmethyl-oxon, O-methoate, dichlorvos and coroxon for 15, 30, and 60 min were performed in the presence of calcium or EDTA. The concentrations of unhydrolyzed oxon which produce 50% ChE inhibitions (IC50) with EDTA or calcium were quantified from standard curves of ChE inhibition. IC50 values in 70 day animals with these compounds did not differ significantly in the presence and absence of calcium, indicating a negligible amount of oxon degraded. This evidence suggests that these compounds are poor substrates for A-esterase hydrolysis. Diazoxon showed a statistically significant increase in the amount of oxon degraded with age, with PND 3, 12, 21, 33, and 70 days exhibiting 4, 34, 109, 132 and 159 pmoles/min/mg tissue, respectively, of product formed. Direct A-esterase hydrolysis detecting the cleavage product of dichlorvos, and O-methoate were performed, and showed no difference in the presence and absence of calcium. The data suggest that in the case of diazoxon toxicity, lower levels of A-esterase activity in young animals, contributes to the greater toxicity of this compound in the young.

#### 431 HEPATIC MICROSOMAL CARBOXYLESTERASE ACTIVITY IN RATS FOLLOWING PRETREATMENT WITH KETONE COMPOUNDS.

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The activity of hepatic microsomal carboxylesterase was determined in rats that were pretreated with one of the following ketone compounds: acetone, 2-butanone, 2-pentanone, 2-hexanone, or pinacolone. These compounds with increasing molecular weight were selected because pinacolone, a leaving group of soman metabolism, had previously been found to enhance esterase activity following pretreatment in guinea pigs (Luttrell and Castle, 1993). Groups of male rats (n = 4-6) were pretreated subcutaneously with 90% LD50 or 90% LD10 (lowest published lethal dose) for each compound and then sacrificed by cervical dislocation 12 hours later. Microsomes were isolated and overall carboxylesterase activity was determined spectrophotometrically. Para-nitrophenyl acetate (0.02 M) was used as the substrate and carboxylesterase activity was calculated by measuring the absorbance change at 420 nm by following release of para-nitrophenol for one minute. Assays were performed in duplicate and enzyme activities expressed in terms of umoles para-nitrophenol/mg protein/minute. Statistical difference (p < 0.05) with the control value is indicated by \*. The data in the table show that pretreatment with ketone compounds of increasing molecular weight results in increasing hepatic carboxylesterase activity. Pretreatment with smaller ketones resulted in inhibition, while pretreatment with larger ketones resulted in enhancement. Luttrell, W E and Castle, M C. Enhancement of hepatic microsomal esterase activity following soman pretreatment in guinea pigs. *Biochem. Pharm.* 1993;46(11):2083-2092.

Dose Group (n)	Carboxylesterase Activity (Mean +/- SEM)
Control (9)	0.182 +/- 0.011
Acetone (6)	0.140 +/- 0.018*
2-Butanone (5)	0.147 +/- 0.018*
2-Pentanone (4)	0.188 +/- 0.088
2-Hexanone (4)	0.227 +/- 0.118
Pinacolone (4)	0.430 +/- 0.188*

#### 432 METABOLISM OF 14C-LABELED 1,3-DICHLOROPROPENE IN FISCHER 344 RATS AND B6C3F1 MICE.

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The metabolism of 1,3-dichloropropene was examined in the male F344 rat and the male B6C3F1 mouse to characterize the dose- and species-dependence differences in *in vivo* fate of this compound. Following oral administration of a low dose of 1 mg/kg or a high dose of 50 mg/kg (rat) or 100 mg/kg (mouse), both test materials were rapidly absorbed and metabolized primarily *via* glutathione conjugation. The major urinary metabolites identified in the rat were the mercapturic acid conjugate of the test material and the corresponding sulfoxide. Glutathione conjugation followed by secondary metabolism afforded the 3-thiopyruvate and 3-thioacetate conjugates as major urinary metabolites in the mouse. Numerous other minor metabolites arising from the glutathione conjugation pathway were also identified in both species. One or more dimercapturic acid metabolites were also found, arising from dual conjugation with glutathione. Several highly polar metabolites were not identified, but were comparable to those obtained as metabolites of the hydrolysis products 3-chloroallyl alcohol and 3-chloroacrylic acid. As previously reported, this compound also undergoes metabolism to CO<sub>2</sub>, accounting for 15-18% and 14% of the administered dose in the rat and mouse, respectively (Dietz *et al.*, 1985, *Toxicologist*, 4, 147).

#### 433 EPOXIDATION OF 1,3-DICHLOROPROPENE: A HIGH DOSAGE PHENOMENON IN MICE.

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1,3-Dichloropropene is an agricultural nematocide. The major routes of metabolism of DCP in rodents are conjugation with glutathione and hydrolysis followed by oxidation to carbon dioxide. Additionally, it has been demonstrated that at IP dosages of 350-700 mg/kg, which are equal to or exceed the reported oral LD50 in mice, DCP may undergo epoxidation (Schneider *et al.*, *Chem. Res. Toxicol.* 11, 1137-44, 1998). The potential for epoxidation of DCP in rats and mice at sublethal dosages was therefore examined. In addition, the formation of the epoxide (DCPO) by liver microsomes from mice, rats and humans in the absence of detoxification enzymes *in vitro* was also evaluated. Following oral administration of 100 mg/kg to F344 rats and B6C3F1 mice, no DCPO was found in the liver or blood of rats or mice at 0-90 min post-dosing (LOD= 5-10 ppb). In contrast, following IP administration of 100 mg/kg a very low level of DCPO was detected in the blood of B6C3F1 mice. IP administration of 700 mg/kg to B6C3F1 or CD-1 mice resulted in a disproportionately higher level of DCPO (>3x LOD), a dosage which was lethal within approximately 1 hr. Serum ALT and AST activities were elevated following IP but not oral administration of DCP to B6C3F1 mice. *in vitro* formation of DCPO was also found to be similar between all three species examined. These data indicate that any appreciable epoxidation of DCP in mice and rats occurs at high, even lethal, dosages and that human microsomes are no more active at forming DCPO *in vitro* than rodents.

#### 434 A MODEL FOR ISOPRENE KINETICS AND METABOLISM IN THREE DIFFERENT SPECIES: RAT, MOUSE AND HUMANS.

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The carcinogenicity of isoprene is known to be species specific. Mice were found to be more susceptible for tumor development than rats. For humans, isoprene is classified as possibly carcinogenic. Similar to the structurally related butadiene, isoprene can be metabolized to a mutagenic di-epoxide. In order to understand the apparent species differences, *in vitro* metabolism rates of isoprene and of its metabolites were measured for rats, mice and humans. P450 activities towards isoprene were measured to estimate the rate of epoxide formation. Epoxide hydrolase and GST activities towards the epoxide metabolites were determined to estimate their inactivation. Largest differences in *in-vitro* rates were observed in the microsomal epoxide hydrolases (mouse<<rat<<human) and in glutathione conjugation of the mono-epoxides (mouse>rat>>human). In order to compare the different species a PBPK model for inhalation exposure of isoprene and the disposition of isoprene as well as the two mono-epoxides and the di-epoxide metabolite was set up. Lung and